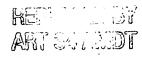
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Claims



- 1. Method for producing a protein comprising the steps:
 - (a) providing a nucleic acid sequence coding for the protein in which a heterologous nucleic acid sequence is inserted on the 3' side of the translation start codon in the correct reading frame, said heterologous nucleic acid sequence being selected such that a stem-loop structure is formed on the 3' side of the translation start codon at a distance of 6-30 nucleotides,
 - (b) providing an expression system suitable for expressing the protein and
 - (c) introducing the nucleic acid sequence according to (a) into the expression system according to (b) under such conditions that the protein is synthesized.
- 2. Method as claimed in claim 1 additionally comprising the isolation of the protein.
- 3. Method as claimed in claim 1 or 2,
 characterized in that,
 the inserted heterologous nucleic acid sequence has a length of up to 201 nucleotides.
- 4. Method as claimed in claim 3,
 characterized in that
 the inserted heterologous nucleic acid sequence has a length of up to 45 nucleotides.

- Method as claimed in one of the claims 1 to 4,
 characterized in that
 the stem-loop structure is formed at a distance of 12-21 nucleotides on the 3'
 side of the start codon.
- 6. Method as claimed in one of the claims 1 to 5,
 characterized in that
 the length of the stem in the stem-loop structure is in the range of 4-12 nucleotides.
- 7. Method as claimed in one of the claims 1 to 6,

 characterized in that

 the region of the heterologous nucleic acid sequence that is on the 5' side of

 the stem-loop structure does not itself form a secondary structure and cannot

 form a secondary structure with the 5' untranslated region of the nucleic acid

 sequence coding for the protein to be produced.
- 8. Method as claimed in one of the claims 1 to 7,

 characterized in that

 the region of the heterologous nucleic acid sequence that is on the 5' side of
 the stem-loop structure and on the 3' side of the ATG start codon has a GC

 content of < 50 %.
- Method as claimed in one of the claims 1 to 8, characterized in that
 an in vitro expression system is used.

- 10. Method as claimed in claim 9,characterized in thata prokaryotic in vitro expression system is used.
- 11. Method as claimed in claim 10,

 characterized in that

 the prokaryotic in vitro expression system comprises lysates of gramnegative bacteria in particular of Escherichia coli or of gram-positive bacteria in particular Bacillus subtilis.
- 12. Method as claimed in claim 9,characterized in thata eukaryotic in vitro expression system is used.
- 13. Method as claimed in claim 12,

 characterized in that

 the eukaryotic in vitro expression system comprises lysates of mammalian

 cells in particular of rabbits, reticulocytes, human tumour cell lines, hamster

 cell lines, or other vertebrate cells, in particular oocytes and eggs of fish and

 amphibia as well as insect cell lines, yeast cells, algal cells or extracts of

 plant seedlings.
- 14. Method as claimed in one of the claims 1 to 8,characterized in thata prokaryotic in vivo expression system is used.
- 15. Method as claimed in claim 14,characterized in thata prokaryotic host cell is used as the expression system.

- 16. Method as claimed in claim 15,

 characterized in that

 a gram-negative prokaryotic host cell, in particular an E. coli cell or a grampositive prokaryotic host cell, in particular a Bacillus subtilis cell is used.
- 17. Method as claimed in claim 14,characterized in thata eukaryotic host cell is used as the expression system.
- 18. Method as claimed in claim 17,

 characterized in that

 a yeast cell, an insect cell or a vertebrate cell in particular an amphibian,

 fish, bird or mammalian cell is used.
- Method as claimed in claim 14,
 characterized in that
 a non-human eukaryotic host organism is used as the expression system.
- 20. Method as claimed in one of the claims 1 to 19,

 characterized in that

 the nucleic acid sequence coding for the protein is provided by cloning,
 recombination or/and amplification.
- 21. Method as claimed in claim 20,characterized in thatthe provision comprises a two-step PCR.

22. Method as claimed in one of the claims 1 to 21,

characterized in that

the nucleic acid sequence coding for the protein to be produced or/and the heterologous nucleic acid sequence at least partially have a codon usage adapted to the respective expression system.

23. Method as claimed in one of the claims 1 to 22,

characterized in that

the heterologous nucleic acid sequence contains a section coding for a purification domain or/and a section coding for a proteinase recognition domain.

- 24. Reagent for producing a protein comprising
 - (a) a nucleic acid sequence that is heterologous to the nucleic acid sequence coding for the protein which can be inserted into the protein-coding nucleic acid sequence in the correct reading frame and which can form a stem-loop structure at a distance of 6-30 nucleotides on the 3' side of the translation start codon, and
 - (b) an expression system that is suitable for producing the protein.